Manual for the use of mating-based Split ubiquitin system "mbSUS"

version A

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Introduction and general information

Abbreviations

X = prey peptide/ORF
Y = bait prey/ORF
N = NubG
C = Cub
PLV = protA-LexA-VP16 peptide
HA tag = hemagglutinin epitope tag

Ade = adenine
His = histidine
Trp = tryptophan
Leu = leucine
Ura = uracil
Met = methionine

X-Gal = 5-bromo-4-chloro-3-indol-b-D-galactosidase
ONPG = o-nitrophenylgalactopyranoside

SC = synthetic complete medium
SD = synthetic dextrose / minimal medium
SSDNA = carrier DNA from salmon sperm

mbSUS package contents

You have received the package with mating-based Split ubiquitin system "mbSUS". This package contains:

- yeast strains THY.AP4 and THY.AP5
- vectors (resuspended in dH_2O, ca. 50 ng/ul)
  - 1 pXNgate21-3HA
  - 2 pNXgate33-3HA
  - 3 pX-NubWTgate
  - 4 pNubWT-Xgate
  - 5 pMetYCgate
- control constructs (resuspended in dH_2O, ca. 50 ng/ul)
  - 6 KAT1-Cub (Arabidopsis K^+ channel, Acc. At5g46240)
  - 7 KAT1-N
  - 11 N-KAT1-3HA

Restriction analysis:

The maps of the vectors and control constructs are shown in the section "Maps of vectors and control constructs". The sequences of the vectors1-5 in genebank format are in the "Appendix".

General information, protocols, maps and sequences of the vectors are included in this manual. For further information please check Obrdlik *et al.*, 2004 and Ludewig *et al.*, 2003.

References and citation

When citing the system, the vectors **pXNgate21-3HA, pX-NubWTgate, pNubWT-Xgate and pMetYCgate,** and the control constructs please refer to the publication **Obrdlik *et al.*, 2004, PNAS 101; 12242-12247.**

When citing the **vector pNXgate33-3HA**, please refer to **C. Cappellaro and E. Boles, University of Frankfurt, unpublished**.

Differences to the vectors published in Obrdlik *et al.*, 2004

The vectors **pXNgate21-3HA** and **pNXgate33-3HA** are based on the corresponding vectors pXNgate and pNXgate in Obrdlik *et al.* (2004). Their additional feature is the **triple HA tag**, which allows the construction of ORF-NubG-3HA and NubG-ORF-3HA fusions. The triple HA tag is readily detectable with anti-HA antibodies. In addition, **pNXgate33-3HA** is a **low-copy CEN** plasmid and produces significantly higher signal/noise ratio when compared to pNXgate in Obrdlik *et al.* (2004).

Please note that the control construct **KAT1-N** has not a triple but **single HA-tag** peptide fused to the C-terminus!

The mbSUS in brief

General information

-The vectors are designed for recombinational *in vivo* cloning in yeast: you need only **one PCR-product** of the gene of interest, which you can insert in any of the Nub or Cub vectors ("*In vivo* cloning into mbSUS vectors" and Fig. 4 and 5).

- Big plus: the linkers in our system contain attB1 and attB2 sites, which are compatible with GATEWAY cloning (Invitrogen). Thus you can use either the PCR products or the resulting Split-ubiquitin constructs for further cloning into any of the GATEWAY destination vectors (Fig. 6). Nevertheless, keep in mind that the mbSUS vectors themselves are **NOT DESTINATION VECTORS**!

-Expression of CubPLV fusions can be optimized since it is under the control of *MET25* promoter. We **routinely** test interactions on **different methionine concentrations**.

-We use two different yeast strains: THY.AP5 is Mat α mating type strain and is designed for the transformation with Nub fusions, the second strain THY.AP4 [Mat a] is designed for transformation with CubPLV fusions. Therefore we can test the interactions by **mating approach** in diploid cells. (see "mbSUS tests protocol" and Fig. 5)

-There are three reporter constructs in our yeast strains: *lexA-HIS3*, *lexA-Ade2* and *lexA-lacZ.* Thus we can select the interacting partners via growth (on -Ade-His media), by white color on non-selective media (+Ade+His) or via ß-galactosidase assays (Fig. 3)

-NubG vectors possess a **3 x HA tag** at their C-termini, it is detectable in most of the Nub fusions tested. This is useful if you want to show, that the Nub fusions are indeed expressed.

- There are also vectors for construction NubWT-X and X-NubWT fusions. Especially **X-NubWT fusions** can be useful since the affinity of NubG for Cub in X-NubG is often very low and hardly shows any interaction (see also below).

- The **empty NubWT-X** gate vector (i.e. soluble NubWT peptide) should also be used as a positive control.

Control constructs (C=Cub, N=NubG)

The system works very well for KAT1 interactions: we use the homomerization of KAT1 as a positive control in our assays. Hence the KAT1-C, KAT1-N and N-KAT1-3HA are included as positive controls. KAT1-N contains a single HA tag at the C-terminus.

NubG-X or X-NubG fusions ?

In general, Cub has higher affinity to Nub fused to the N-termini of prey (Nub-X fusions) than to Nub fused to C-termini of prey (X-Nub fusions).

When testing interactions with a set of X-NubG fusions (e.g. KAT1-Cub with X-NubG fusions) the signal/background ratio is usually very high. However you may not be able to detect "weak" interactions.

When using NubG-X fusions it is important to test, that your CubPLV fusions do not show background activities in growth assays with different NubG-X fusions (false positives)! It is crucial to use **several different** NubG-X fusions as negative controls! An empty pNXgate33-3HA vector (soluble NubG peptide) is not enough!

If you observe background problems you can perform **quantitative β-galactosidase assays** (measuring lacZ activity, **Obrdlik *et al.*, 2004, Ludewig *et al.*, 2003**), which can distinguish between false and true interactions. Alternatively try to adjust a proper methionine concentration in the medium.

Important!
For *in vivo* cloning of the PCR product combined with direct interaction tests use pools of 5-10 independent clones as described in the protocol for screening "mbSUS tests protocol". In this way it is possible to suppress the effects of non-functional mutants. Such mutations can be caused by the RT-PCR as well as by the recombination events during *in vivo* cloning (rare but possible mutations in the vicinity of homologous regions).

The sensitivity of the methods for detection of interaction:
The growth assays via selection on media -Ade-His-Trp-Leu-Ura is the most sensitive one. The detection of interactions via lacZ-activity by X-Gal assays and liquid ONPG assays is not as sensitive but liquid assays are useful for quantitative analysis. The detection of interactions by monitoring the white (interaction) and red (no interaction) color of diploid cells (Fig. 3) is the least sensitive and can be used only in addition to the other detection methods.

Check-list before you start your experiments with mbSUS

1) In order to check CubPLV fusions, use the empty pNXgate33-3HA and pNubWT-Xgate vectors as negative and positive controls, respectively.

2) It is not sufficient to use the empty pNXgate33-3HA vector as a negative control. Use also NubG fusions of several other membrane proteins as negative controls!

3) If you want to construct X-Nub fusions, consider also creating X-NubWT fusions. X-NubWT fusions can provide specific results if you analyze the interactions via quantitative lacZ assays (but do not forget negative controls from 2!).

4) For the production of a B1-ORF-B2 inserts by PCR ("*In vivo* cloning into mbSUS vectors" and Fig. 4) it is better to start from a sequenced DNA template. Using this you will reduce the risk of mistakes produced during PCR.

Protocols

This section contains **protocols adapted for mbSUS**. General protocols and the preparation of general media and chemicals should be performed as described in "Methods in Yeast Genetics" (Adams *et al.*, Cold Spring Harbor Lab. Press) and in "Current Protocols in Molecular Biology" (Ausubel *et al.*, John Wiley & Sons Inc.).

In vivo cloning into mbSUS vectors pNXgate33-3HA, pXNgate21-3HA, pNubWT-Xgate, pX-NubWTgate and pMetYCgate

Principle of the *in vivo* cloning is described in Obrdlik *et al.*, 2004 and in Fig. 4 of this manual.
For *in vivo* cloning into mbSUS vectors the ORFs have to be flanked by B1 and B2 linkers via PCR. In parallel the vector pMetYCgate and the Nub vectors have to be restricted with PstI/HindIII and with EcoRI/SmaI, respectively.
Both, the B1-ORF-B2 PCR product and the appropriate linear vector are used to co-transform either THY.AP4 or THY.AP5 yeast (Fig. 4 and 5). Homologous recombination between B1 and B2 sequences of the B1-ORF-B2 and of the linear vector produces circular vector harboring the ORF. Transformants are selected on -Leu (CubPLV fusions) or on -Trp (Nub fusions).

1): B1 and B2 linker sequences of the vectors

Linker B1

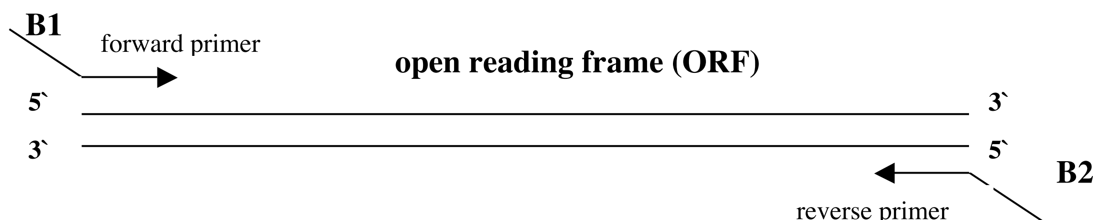
```
                   attB1
aca agt ttg tac aaa aaa gca ggc tct cca acc acc atg
 T   S   L   Y   K   K   A   G   S   P   T   T  Met
```

Linker B2

```
                   attB2                         BamHI
tac cca gct ttc ttg tac aaa gtg gtt ggt ggt ggc gga tcc ggt gga ggt gga tca
 Y   P   A   F   L   Y   K   V   V   G   G   G   G   S   G   G   G   G   S
```

The underlined B1 and B2 nucleotid sequences are crucial for GATEWAY cloning and may not be changed according to codon usage rules (even if the corresponding amino acid is the same)! (Invitrogen; Hartley et al. (2000) Genome Research 10: 1788; Walhout et al. (2000) Science 287:116).

2): Primer design for in vivo cloning into mbSUS vectors



B1-forward primer (5´strand)

```
                        B1-linker                start-ORF
aca agt ttg tac aaa aaa gca ggc tct cca acc acc atg xxx-5´strand cDNA
```

B2-reverse primer (3´strand)

```
                        B2-linker
tcc gcc acc acc aac cac ttt gta caa gaa agc tgg gta xxx-3´strand cDNA w/o stop!
```

3): Enzymes for the cleavage of the mbSUS vectors for *in vivo* cloning

pXNgate3HA, pX-NubWTgate:	*Eco*RI & *Sma* I
pNXgate3HA, pNubWT-Xgate:	*Eco*RI & *Sma* I
pMetYCgate:	*Pst* I & *Hind* III

MbSUS tests protocol

See also Fig. 3, 4 and 5

Recombinational *in vivo* cloning:

1. Cut the vectors with restriction enzymes as described in "*In vivo* cloning into mbSUS vectors". Purify the fragments via agarose gel.
2. Make a B1-ORF-B2 insert by PCR using primers described in "*In vivo* cloning into mbSUS vectors". Purify the PCR fragments via affinity columns.
3. Co-transform a linear vector and the B1-ORF-B2 insert into either THY.AP4 (Cubs) or THY.AP5 (Nubs) as described in "LiAc Transformation". Do not forget to transform the linear vector alone (negative control!). Plate on appropriate media without Leu (Cub vectors in THY.AP4) or without Trp (Nub vectors in THY.AP5).

Mating:

4. Collect **5-10 independent clones** and mix them in 0.1 ml dH2O. Use 20-100 µl of this suspension to inoculate 5ml of the appropriate SC media without G418 and 5 ml with G418 (starting cultures should be only slightly turbid).
5. Grow cells over night to the stationary phase (stock culture). Cells carrying vectors with inserts should not grow on G418.
6. Concentrate 1ml of the cultures by centrifugation in a final volume of 200µl YPD. 200 µl are enough for 13 crossings. For higher number of crossings use a higher volume of the culture (e.g. for 26 crossings concentrate 2ml culture in 400 µl YPD). Mating and replica plating is more efficient if the final suspension is not too thin!
7. Mix 15 µl of the appropriate mating types for each cross. You can use microtiter plates for large number of interactions.
8. Drop 4 µl of the mixed suspensions on a YPD-plate (this should give a patch of about ϕ7mm). It takes a while for the liquid to be adsorbed. For that reason plates should be pre-dried under a hood.
9. Mate the cells for 6-8h at 28°C.
10. Replica plating on SC/+Ade+His; make minimum 2 replicas of each YPD plate
11. Selection of diploid cells (2-3 days incubation at 28°C).

Interaction growth tests:

12. Replica plating of the cells on 4 different plates (synthetic minimal medium)
    a) + 0 mM methionine
    b) + 75 µM methionine
    c) + 150 µM methionine
    d) + 400 µM methionine
13. In addition to replica plating it is recommended to streak out the colonies on synthetic minimal medium with different methionine concentrations to check for growth properties!
14. After two days at 30°C start to record the growth.

X-gal tests:

15. Replica plating of the cells on different plates
    (synthetic minimal medium +ADE, +HIS,)
    a) + 0 mM methionine
    b) + 150 µM methionine
16. After 2-3 days test the β-galactosidase activity by "X-Gal Overlay assay".

Synthetic minimal medium (SD)

2% w/v Glucose
0,17% w/v yeast nitrogen base *without* ammonium sulfate and amino acids
0,5% ammonium sulfate
pH adjusted to 6-6,3 with NaOH

Synthetic complete medium (SC)

like SD medium but add to 1L medium
1,5g of "AHTLUM"-drop out (-Ade, -His, -Leu, -Trp, -Ura, -Met)
add appropriate chemicals for auxotrophy selection (see below)

AHTLUM minus drop-out

standard protocol for drop-out, but without Adenine, Histidine, Leucine, Tryptophan, Uracil, Methionine

Chemicals for auxotrophy selection

all dissolved in dH2O and sterilized by filtering

	chemical	stock conc. (g/100ml)	vol (ml) stock for 1L medium	storage
A	adenine sulfate	0.2	10	RT
U	uracil	0.2	10	RT
T	L-tryptophan	1	2	4°C
L	L-leucine	1	10	4°C
H	L-histidine HCl	1	2	4°C
Met	L-methionine	1	2	4°C

all these can be added to the media before autoclaving !!!

Verifying detected interactions

The interactions identified in the directed mbSUS tests with the in-vivo cloned ORFs (Fig. 5 and "mbSUS tests protocol") have to be verified.

1) isolate the plasmid DNA form yeast ("Lazy-bones method for rapid release of plasmid DNA from yeast")

2) Amplify the plasmid DNA in *E.coli*.

3) Isolate the plasmid DNA from *E.coli* and verify the expression cassette by restriction analysis and by sequencing

4) Use the verified plasmid DNA instead of B1-ORF-B2 and linear vectors for mbSUS interaction tests as described "mbSUS tests protocol".

LiAc transformation of yeast

(modified protocol of Gietz & Schiestl, 1995)

Work all the time under strictly sterile conditions!

Making competent cells

1. Incubate one colony of THY.AP5 and THY.AP5, each at 28°C for ca. 24 h (shaking in 5 ml YPAD)

2. Early in the morning: Inoculate 100 ml pre-warmed YPAD with the pre-culture to an OD_{600} 0.08-0,1.

3. Incubate at 28°C and 180-200 rpm till OD_{600} 0.5-0,6.
Important: Minimal incubation time = time necessary for 2-3 duplications!

4. Place cells in sterile tubes (FALCON), centrifuge at 2.500 x g for 5 min (20°C).

5. Remove the medium, re-suspend pellets in 5 ml sterile ddH_2O each and re-centrifuge again as described above.

6. Remove ddH_2O, re-suspend each pellet in 2,5 ml LiAc/TE, pool the suspensions together and mix carefully.

7. Centrifuge (5 min; 2.500 x g, 20°C) and remove the supernatant.

8. Re-suspend the pellet carefully in 0.5-0.8 ml LiAc/TE, let the suspension incubate for 30min at RT (competent cells!). If you use the cells later than 30 min after this step than keep them at 4°C.

Transformation

before starting: boil the SSDNA for 3 min and chill it on ice immediately

9. For each transformation add in the following order:

-20 μl carrier-SSDNA (5 or 10 mg/ml)
-20 μl DNA-mix: = 1x TE buffer and DNA
either: plasmid alone (0,1 - 10 μg)
or: linear plasmid (100 ng) plus insert (>100 ng), (molecular ratio of vector vs insert at least 1:10)
! mix well !

- 4,5 μl 1M LiAc
! mix well !

-50 μl competent cells, **!mix well!**
-300 μl PEG/LiAc mix (prepare it fresh each time!!!) and **mix well!!!**

13. Incubate shaking for 20 min at 30°C (thermo-mixer)

14. Heat-shock in 42°C-waterbath for 20 min (keep the time!!!)

15. Centrifuge at 6000 - 8000 rpm for 1 min and *carefully* remove the supernatant with a micropipette

16. Resuspend the pellet *carefully* in 100μl sterile dH_2O (or sterile 1x TE buffer) with the pipette.

17. Streak the transformation on appropriate selective media (The media on petri dishes should be dry!).
For transformation of *THY.AP4* with Cub constructs: SC/+AHTU
For transformation of *THY.AP5* with Nub constructs: SC/+AHL

!!! For some purposes the transformation efficiency may be too high and it will be difficult to isolate single colonies from the plates! In that case resuspend the cells (step 16) in 1ml of sterile dH_2O and streak out 100µl on the plate

18. Grow for 2-4 days at 28°C (30°C) on selective media.

Stock solutions:

1M LiAc:
LiAc in ddH_2O, pH not adjusted!, sterilize by filtering

10 x TE:
100 mM Tris Cl,
10 mM EDTA, pH 7,5; adjust with NaOH

50% PEG 3350 (Roth) or PEG 4000 (Fluka)
sterilize by autoclaving

5mg/ml salmon sperm DNA (SSDNA), in 1xTE
make aliquots and keep in -20°C

2 x 100 ml sterile ddH_2O

chemicals for auxotrophy selection:
all solutions are in ddH2O and sterilized by filtering:

	amino acid	stock conc. (g/100ml)	vol (ml) stock for 1L medium	storage
A	adenine sulfate	0.2	10	RT
U	uracil	0.2	10	RT
T	L-tryptophan	1	2	4°C
L	L-leucine	1	10	4°C
H	L-histidine HCl	1	2	4°C
Met	L-methionine	1	2	4°C

all these can be added to the media before autoclaving !!!

Media:

YPAD:

add 10 ml of sterile adenine stock to YPD media

AHTLUM minus drop-out:

standard protocol for drop-out, but without Adenine, Histidine, Leucine, Tryptophan, Uracil, Methionine

SC- (*synthetic complete medium*):

for 1L:
1,7 g yeast nitrogen base ***w/o amino acids and ammonium sulfate***
5,0 g $(NH_4)_2 SO_4$
20 g glucose
1,5 g of *AHTLUM minus* drop-out
20 g Oxoid Agar; adjust pH at 6,0-6,3 with NaOH

Add the appropiate amount of auxotrophy selection chemicals. Leu, Trp, Met, Ade, His and Ura can be added to the medium before autoclaving.

SD- (*synthetic dextrose minimal medium*):

the same protocol as SC-medium, but without the *AHTLUM minus* drop-out

Solutions:

LiAc/TE
1 ml 10 x TE
1 ml 1M LiAc
8 ml ddH2O

PEG/LiAc mix:
0,5 ml 10 x TE
0,5 ml 1M LiAc
4,0 ml 50% PEG

X-Gal assays

Overlay assay

1): Dissolve 0.25g agarose in 50ml Z-buffer (pH 7.2) in the microwave. Do not overheat, otherwise precipitation may occur! 50ml is enough for 3-4 petri dishes.

2): Cool down the solution to 50°C and add 1ml of 10% SDS and 1ml of X-Gal solution (40mg/ml in DMF). The final concentration of X-Gal is 0.8mg/ml, for weak interactions one can also use final concentration of 2mg/ml.

3): Pour the solution carefully over the plate. Incubate at 37°C for 5min to several hours.

Solutions

Z-buffer

Na_2HPO_4 x $2H_2O$ 10,68g/L (60mM) **or**: Na_2HPO_4 x 7 H_2O; 16.1 g/L (60 mM)

NaH_2PO_4 x H_2O 5.5 g/L

KCl 0.75 g/L

$MgSO_4$ x $7H_2O$ 0.246 g/L

adjust to pH 7.0 and autolave

40mg/ml X-Gal, in DMF,

store at -20°C

10% SDS

Filter assay

1): Cut nitorcellulose filter under sterile conditions and place it (use sterile forceps) on a selective media plate.

2): Streak the clones on the filter and incubate the petri dish at 30 °C for 2-4 days.

3): Remove the filter carefully from the plate and place it for 5-10 sec. in liquid N_2. Take it out and let it thaw at RT for 1 min.

4): Repeat the step 3).

5): Place the filter with colonies facing up on Whatman paper soaked with 2ml of freshly prepared Z-buffer/X-Gal solution (in a petri dish). Incubate closed petri dish in the dark for 30 min to ON (RT or 30°C)

Solutions:

Z-buffer

described above

X-Gal solution

80 mg/ml in DMF

each time freshly prepared: Z-buffer/X-Gal solution

100 ml Z-buffer

0.27 ml β-mercaptoethanol

1.67 ml X-Gal solution (final X-Gal conc. ca. 1.4 mg/ml)

"Lazy bones" method for rapid release of plasmid DNA from yeast

(Adapted from Kaiser et al., Methods in yeast Genetics, CSHL Press, 1994)

1: Incubate one yeast colony at 28°C in 5ml YPD (ON) or in selective medium (1-2 days)

2: Pellet 1.5ml of ON yeast cell culture at 5000xg for 5 min. Discard the supernatant.

3: Add 0.2ml of the DNA release solution and resuspend the pellet.

4: Add 0.2ml phenol:chloroform:isomylalcohol (25:24:1) and 0.3ml of acid washed glas beads.

5: Vortex at high speed for 5-10 min.

6: Centrifuge for 5 min at RT and full speed (Eppendorf centrifuge).

7: Transfer 120µl the aqueous layer (the upper layer) to a new tube. Precipitate the DNA with 1xVol isopropanol and wash with 1xVol 70% EtOH. Resuspend the pellet in 30µl dH2O.

8: To transform chemo-competent E.coli use 5-10µl of the DNA. For electro-competent cells use 1µl of the DNA.

Solutions

DNA release solution:

2% Triton X-100
1% SDS
100mM NaCl
10mM Tris HCl (8.0)
1mM Na-EDTA
! do not autoclave !

Acid washed beads:

Use 250-500 micron beads (0,7-1,0mm should also work), work under a fume hood !!!

Wash 100ml glass beads in 200ml conc. HCl (stir them carefully from time to time) and let them stand ON in a fume hood.
Discard the HCl and wash the beads several times with dH_2O, to the pH >5.0
(if the pH is still below pH 3.0 after several washing steps, make the last wash with 20mM Na_2CO3, pH 7.0).
Dry the beads in a glass beaker at 70°C ON and transfer them into a bottle.

Figures

THY.AP4 and the CubPLV vector *pMetYCgate*

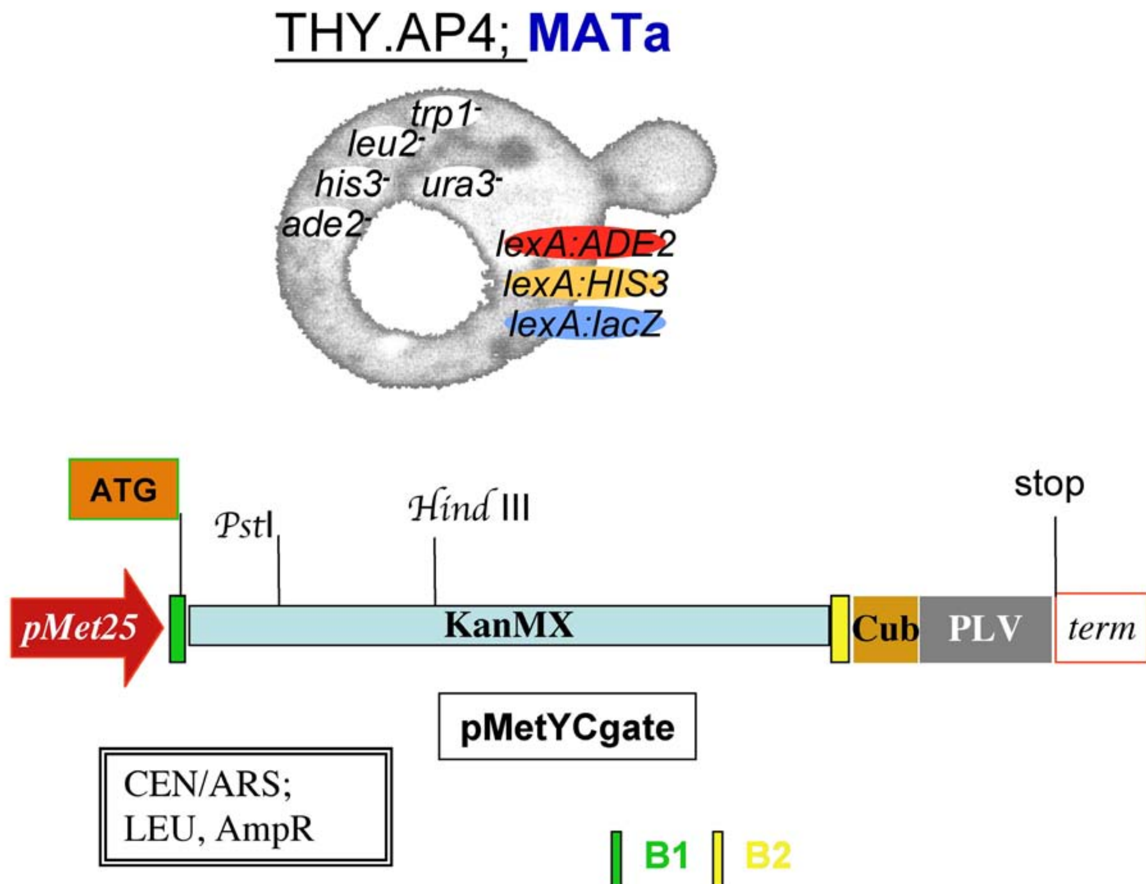


Fig. 1: Reporter yeast strain THY.AP4 and expression cassette of the vector pMetYCgate. pMetYCgate is suited for "Y-CubPLV" fusions of bait peptides "Y". B1-KanMX-B2 is identical with the B1-KanMX-B2 cassettes of the Nub vectors (Fig. 2). Promoter is red, *term* marks the terminator, the linkers B1 and B2 are green and yellow, respectively. Marked restriction sites are used to produce linear vectors for *in vivo* cloning. „*ATG*“ and „*stop*“ mark the start and the stop codon in the expression cassette. Amp^R refers to ampicillin resistance cassette. *CEN/ARS* refers to a low-copy yeast origin of replication. For more details on the pMetYCgate sequence see the appendix.

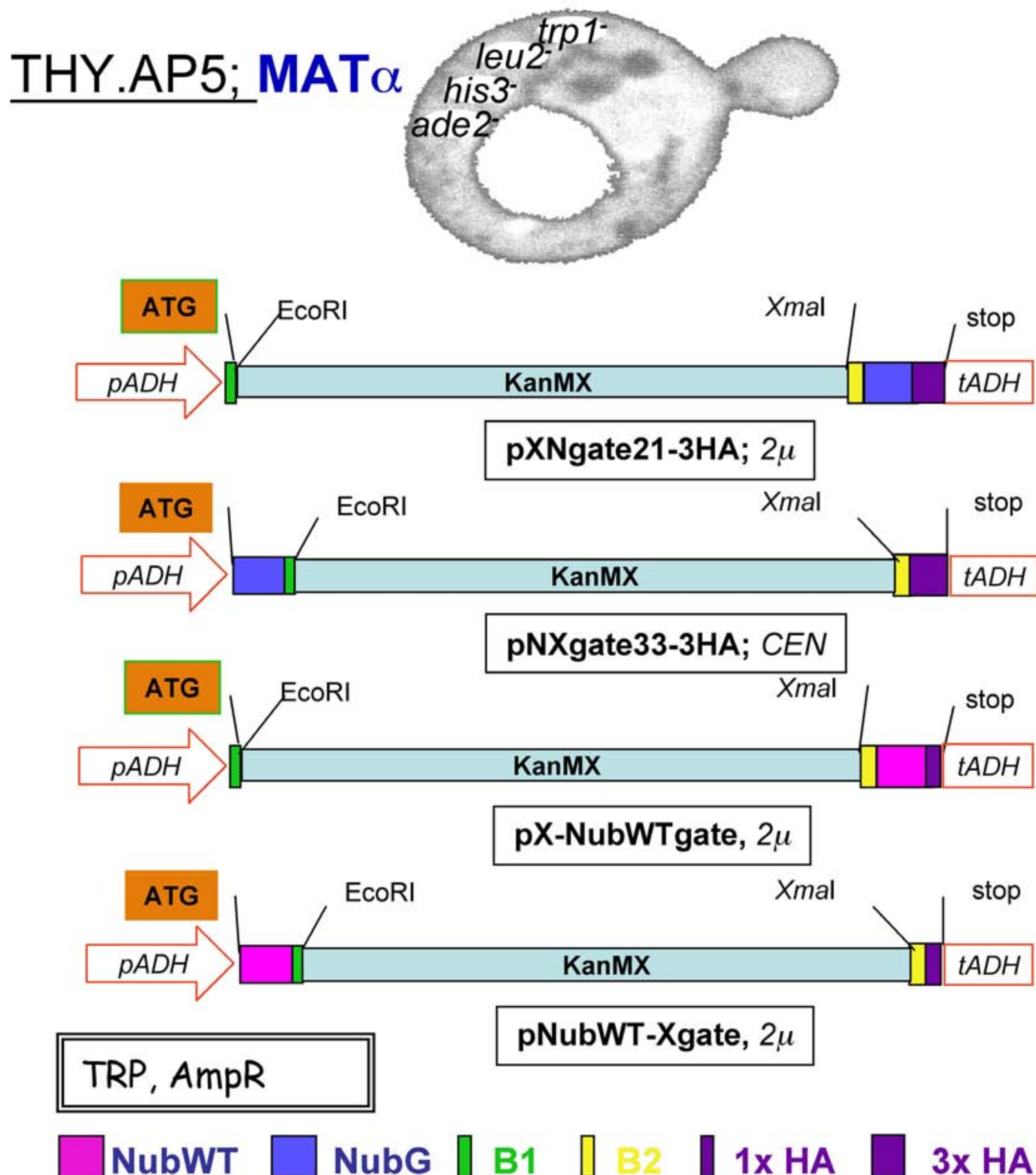


Fig. 2: Yeast strain THY.AP5 and the expression cassettes of the Nub vectors. Nub vectors are suited for ”X-Nub” or “Nub-X” fusions of prey peptides “X”. All B1-KanMX-B2 cassettes are identical (see also Fig. 1). pADH is the ADH1 promoter, tADH marks the ADH1 terminator, NubG, NubWT, HA tag and the linkers B1 and B2 are shown below. Marked restriction sites are used to produce linear vectors for *in vivo* cloning. „*ATG*“ and „*stop*“ mark the start and the stop codons in the expression cassettes. Amp^R refers to ampicillin resistance cassette. 2μ refers to a high-copy, *CEN* to a low-copy yeast origin of replication. All vectors carry TRP1 and AmpR selection markers. For more details on the vector sequences see the appendix.

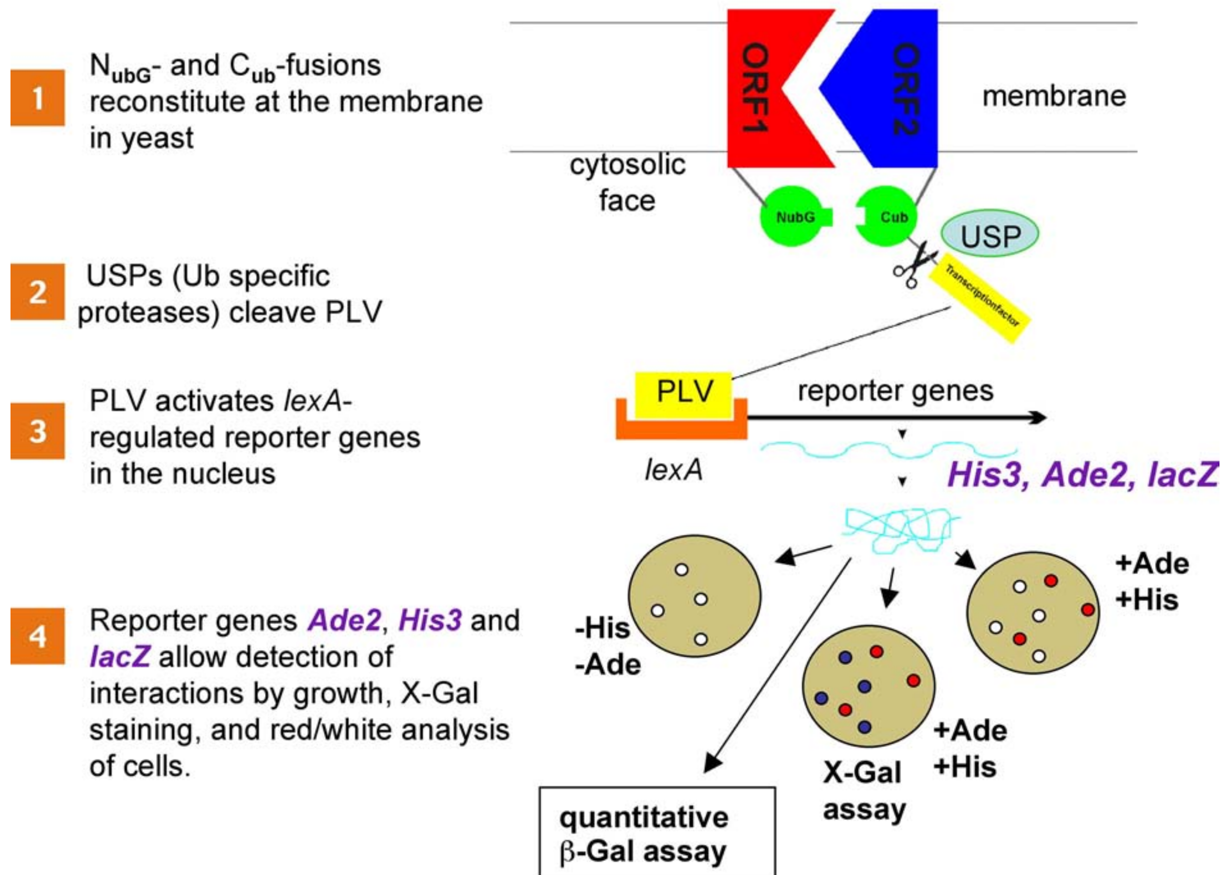


Fig. 3: Detection of interactions with mbSUS. To enable interaction-dependent cleavage of the PLV peptide, NubG and Cub have to be on the cytosolic face of the membrane. The cleaved PLV transcription factor diffuses into the nucleus, binds lexA-regulated promoters and activates the reporter genes. Activation of the reporter genes ***Ade2*** and ***His3*** allows selection of interactions via growth. In addition, ***lacZ*** allows detection of interactions by X-Gal staining and ***Ade2*** enables red/white selection of interacting proteins (white = interaction, red = no interaction). LacZ activity can also be measured with quantitative β–Gal assays.

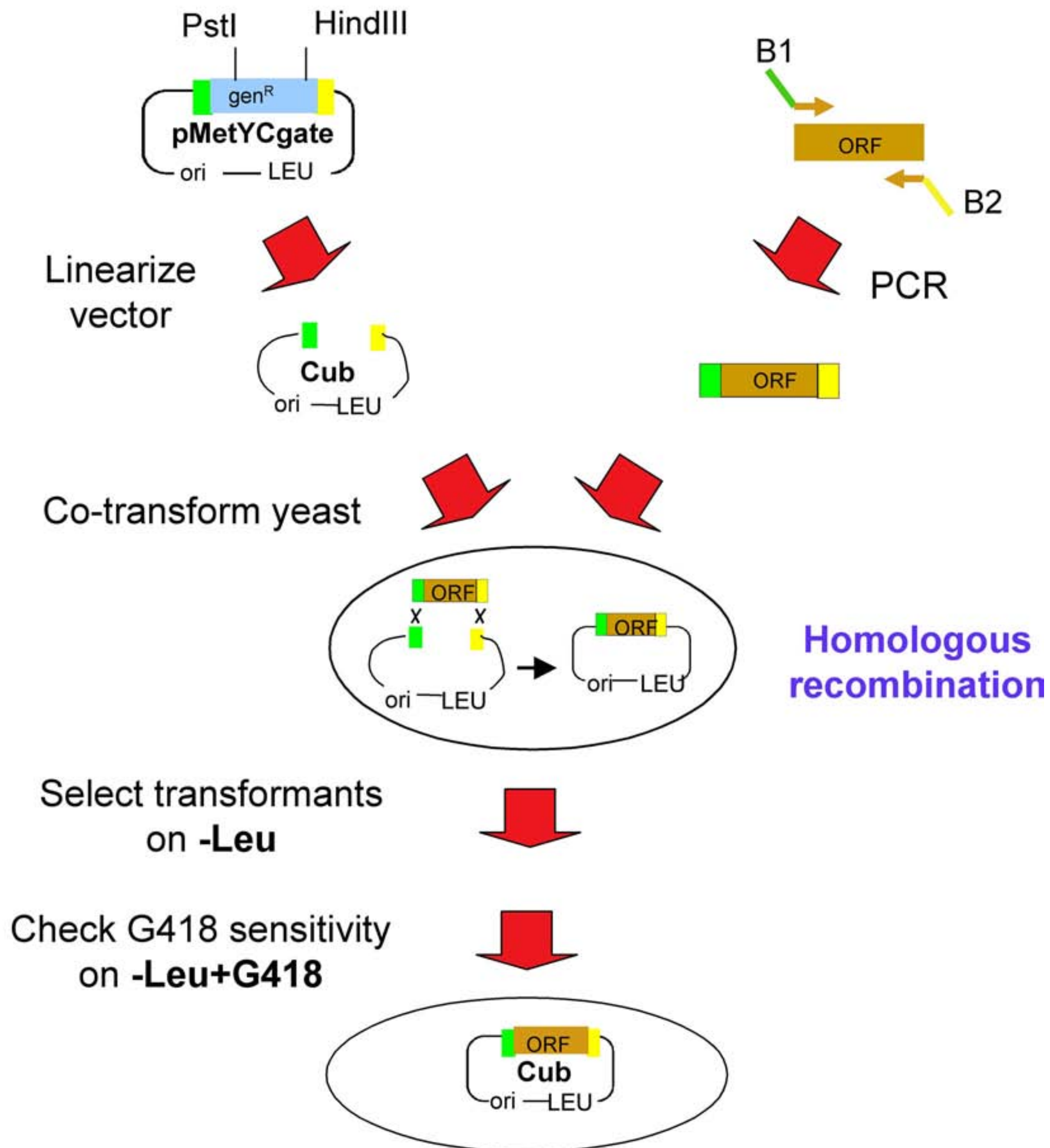


Fig. 4: Principle of *in vivo* cloning into mbSUS vectors. The figure shows the construction of CubPLV fusions. Nub fusions are constructed in similar way using linear pXNgate21-3HA, pNXgate33-3Ha, pX-NubWTgate or pNubWT-Xgate vectors.

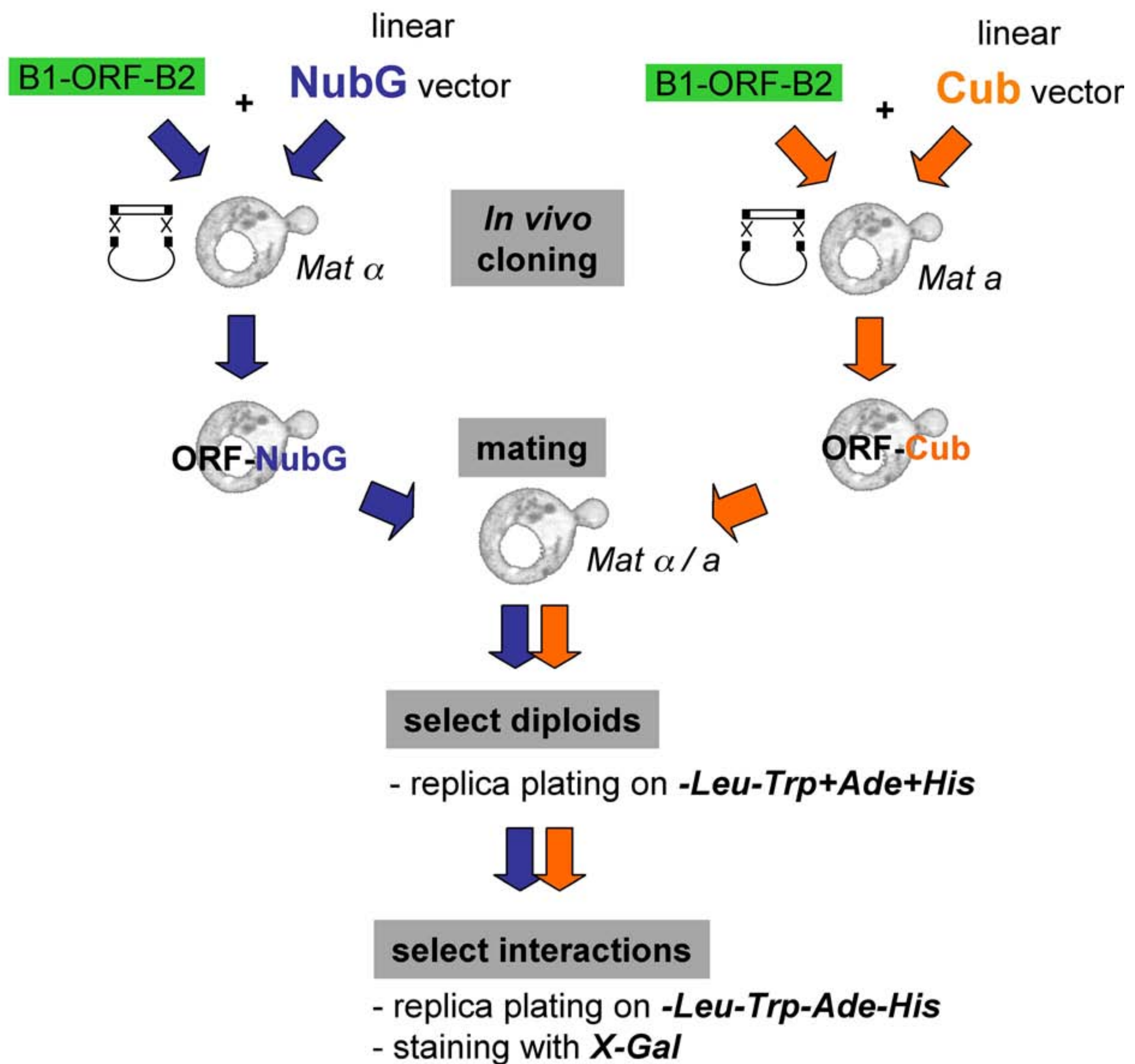


Fig. 5: Flow chart of mbSUS interaction tests. Mat α strain is THY.AP5, Mat **a** strain is THY.AP4. Leu, Trp, Ade and His is leucine, tryptophan, adenine and histidine.

mbSUS as an entry point for GATEWAY cloning

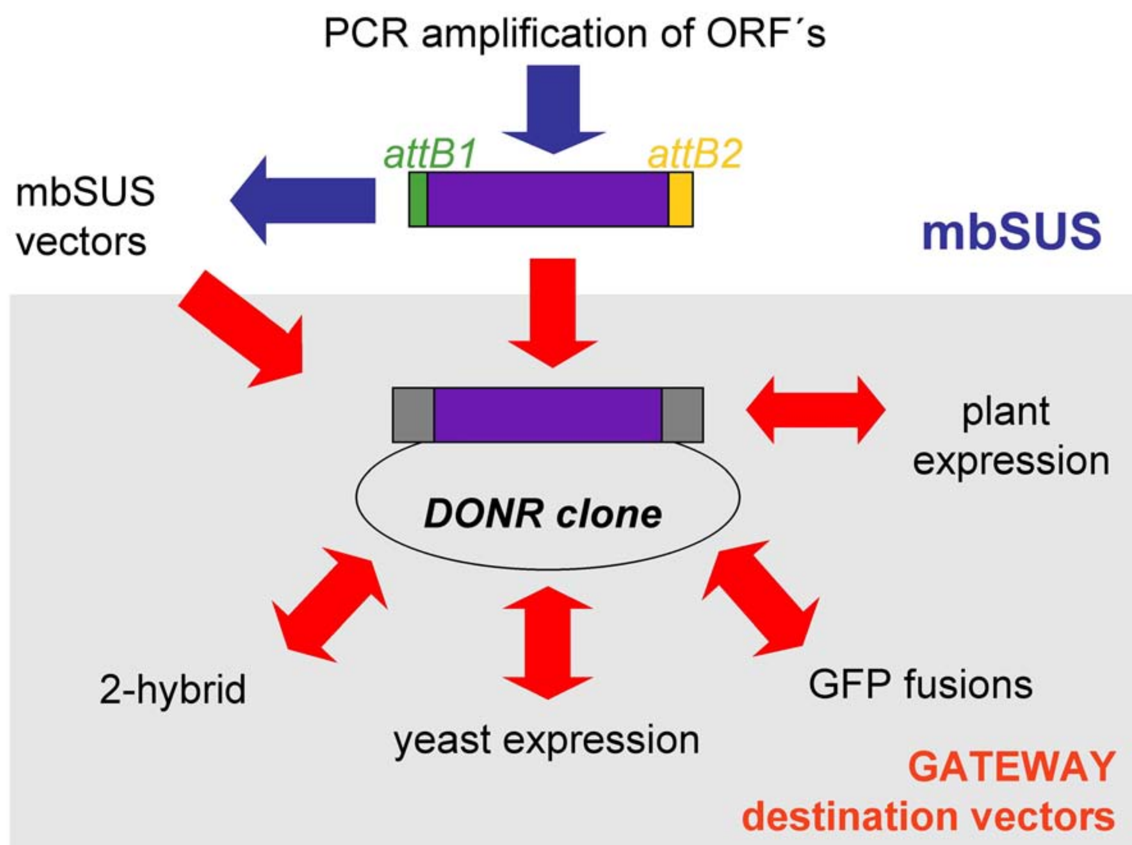
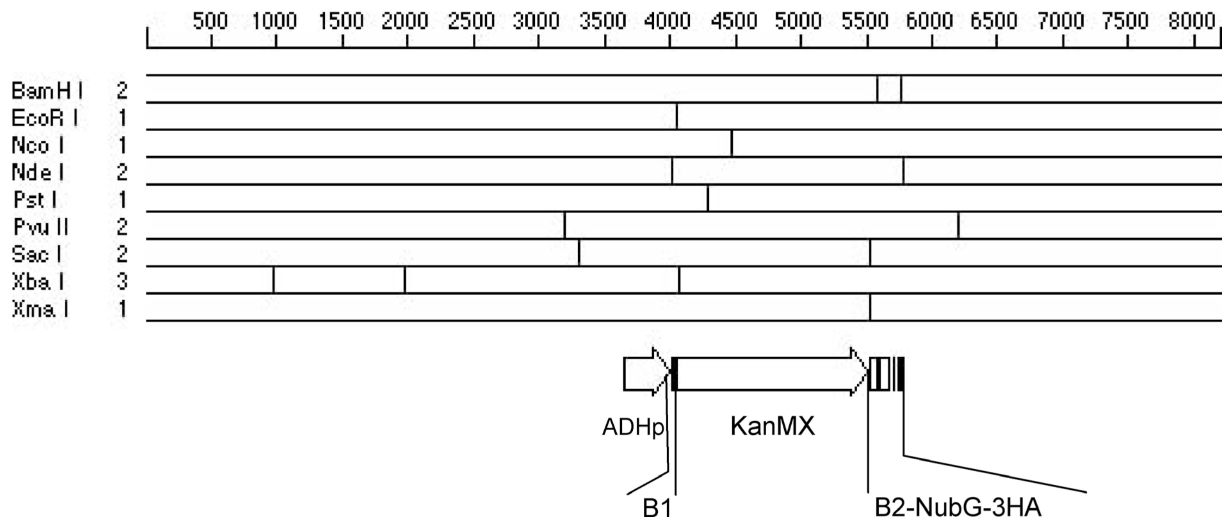


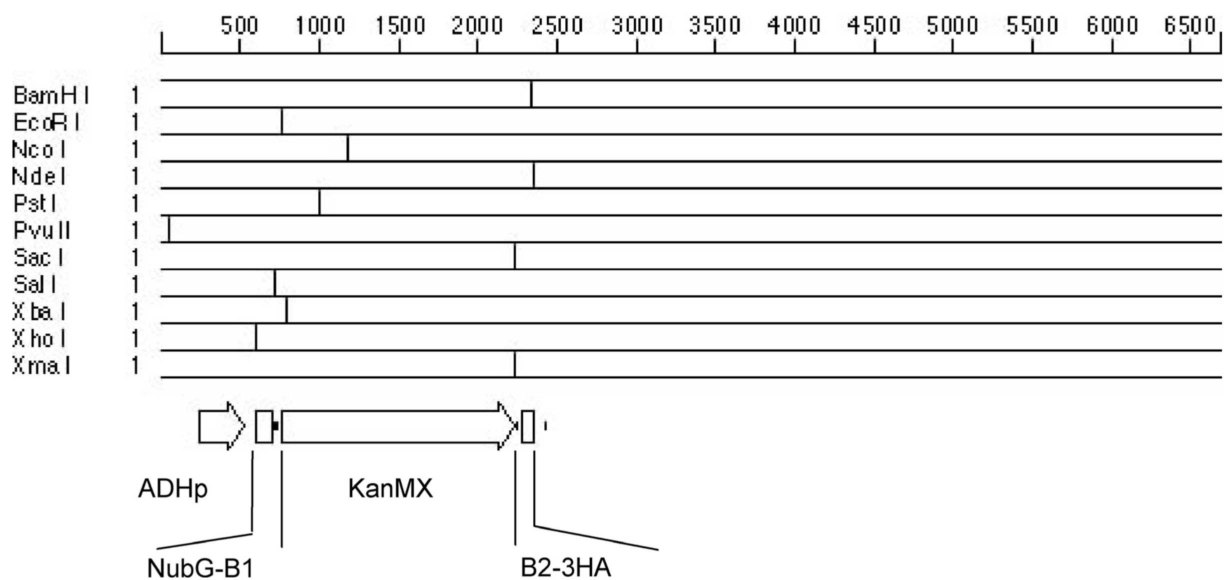
Fig. 6: mbSUS as an entry point for versatile analysis of membrane proteins. B1-ORF-B2 PCR products as well as the mbSUS fusions can be used as entry points to subclone the inserts into different GATEWAY destination vectors. DONR marks the pDONR vectors of GATEWAY (Invitrogen). Please note that the mbSUS vectors are NOT DESTINATION VECTORS and thus are not suitable for direct cloning via GATEWAY!

Maps of vectors and control constructs

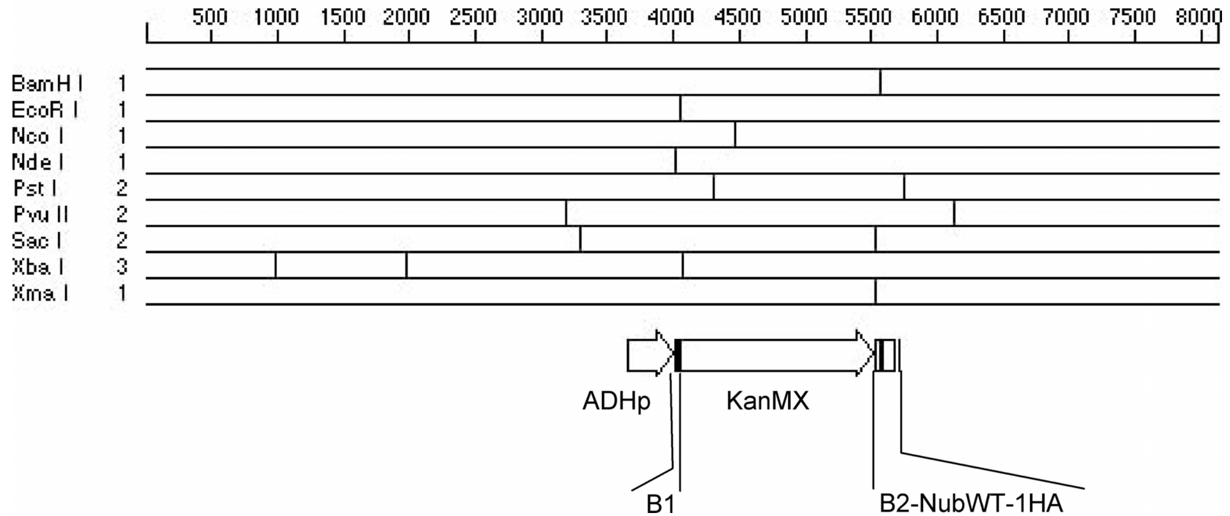
pXNgate21-3HA, 8194 bp
(TRP1, AmpR, 2μ)



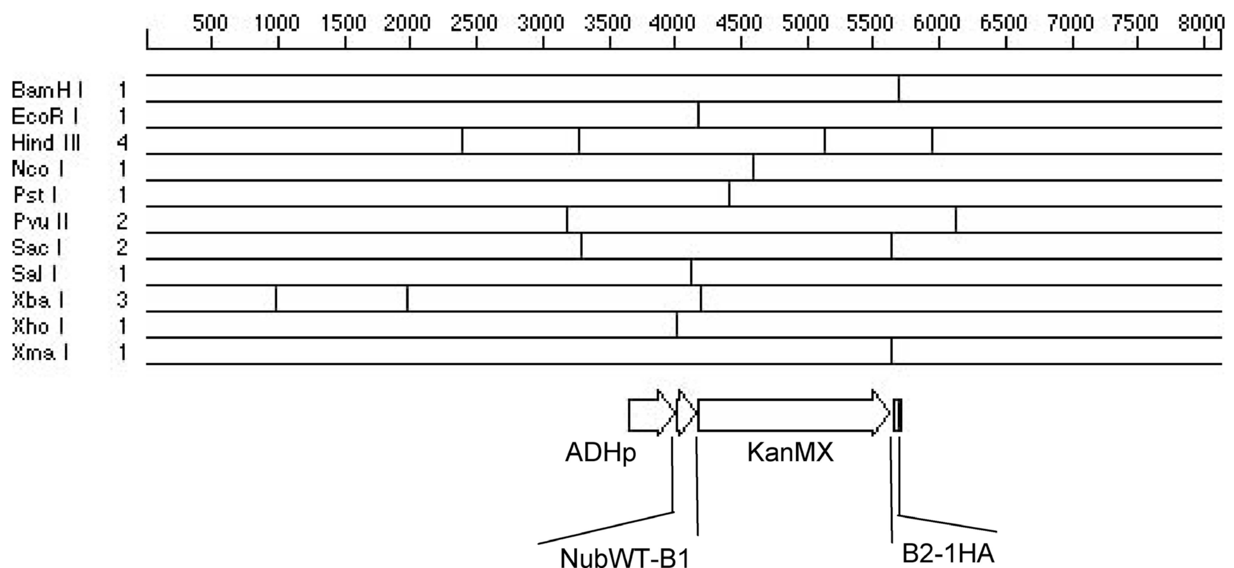
pNXgate33-3HA, 6682 bp
(TRP1, AmpR, CEN)



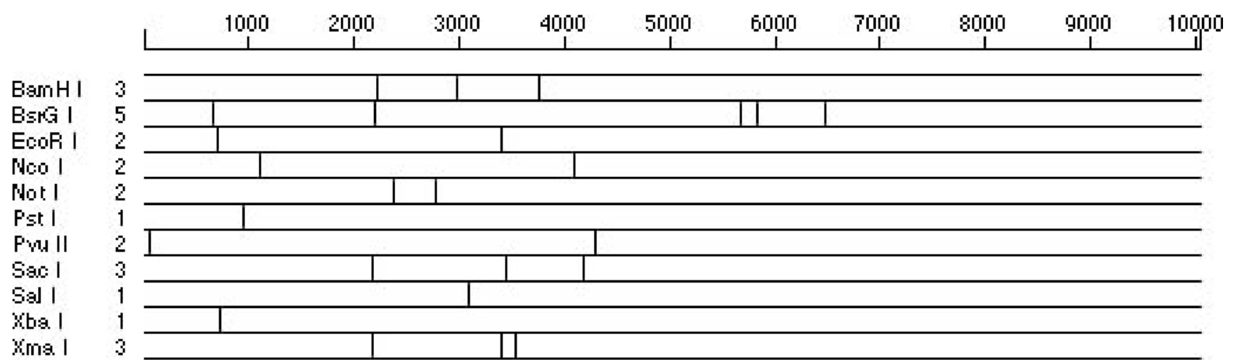
pX-NubWTgate, 8120 bp
(TRP1, AmpR, 2μ)

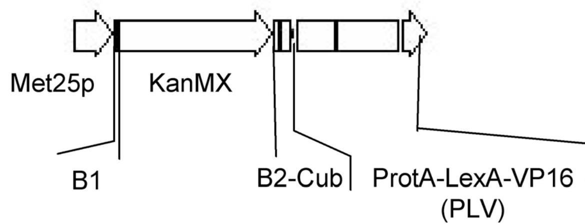


pNubWT-Xgate, 8118 bp
(TRP1, AmpR, 2μ)

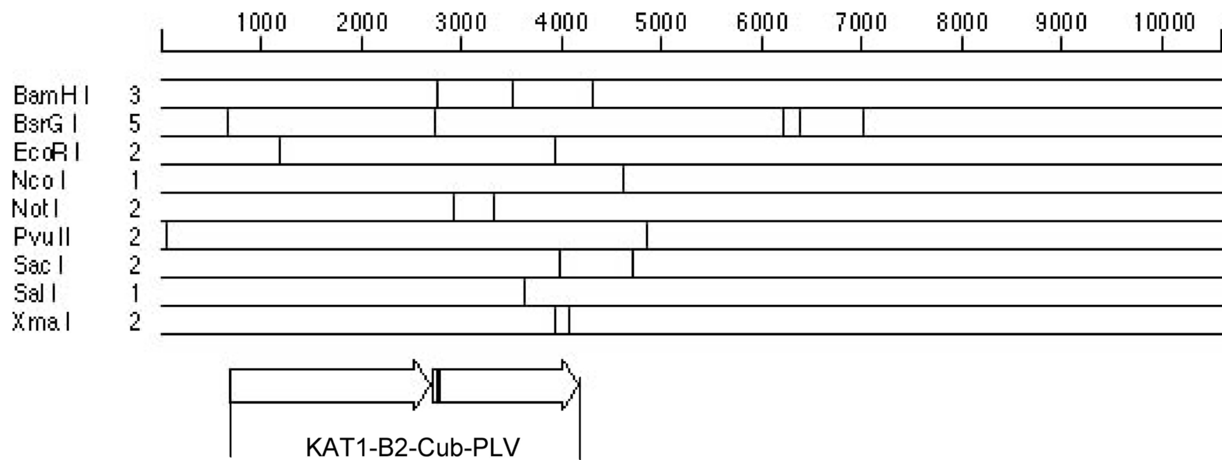


pMetYCgate, 10033 bp
(LEU2, AmpR, CEN/ARS)

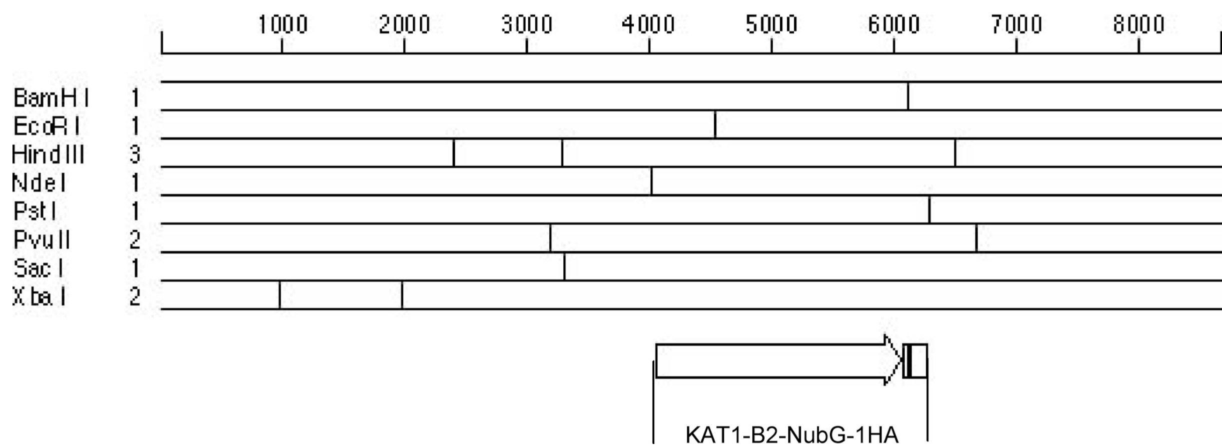




KAT1-C, 10580 bp
(LEU2, AmpR, CEN/ARS)



KAT1-N, 8667 bp
(TRP1, AmpR, 2μ)



N-KAT1-3HA, 7229 bp
(TRP1, AmpR, CEN)